Long term IRT population data to calculate 99.5th and 99.9th centiles at GOSH

Introduction

There are three cut-offs for the CF screen: cut-off 0 (set at approximately 10 ng/ml below cut-off 1), cut-off 1 (99.5th centile) and cut-off 2 (99.9th centile).

The sensitivity and specificity of the CF screen are crucially dependent on the performance of the IRT assay. In particular, if cut-off 1 is set too low, too many samples will be sent for mutation analysis and there will be a disproportionate increase in the number of unaffected carriers detected.

The IRT assay is a particularly difficult assay to control as human blood contains a variety of trypsinogen species which react differently with the various antibodies used for immunoassay.

Monitoring of the population distribution is necessary to ensure that cut-off 1 and cut-off 2 correctly identify the 99.5th and 99.9th centile respectively. However, newborn screening laboratories currently differ in their procedures for reviewing and updating the 99.5th centile.

In a recent audit involving all UK laboratories, responses from participants revealed significant variation in the assigned IRT centiles with same kit lots. The current Buddy group systems did not seem to provide the accurate determination of IRT centiles as recommended in the initial work by RJP and AJM[1]

The review of our IRT data using the last 6 kit lots, spanning 3 years is described.

Methods

Query

A query was used to extract all 425 150 samples between 01/01/2011 and 12/05/2014.

Exclusion criteria

Samples were excluded as follows:

Exclusion reason	Samples excluded	Samples Remaining
Test patients	3	425 147
No IRT result	15 283	409 864
– Parent refusal		
– Contaminated		
– Insufficient		
– Too old		
– Too young		
– No NHS number		
– Moved out of area		
– Died		
– Duplicate card		
– Expired card		
IRT result available but unsuitable	93	409 771
– Too old		
– Too young		
– Insufficient		
Replicates failed	840	408 931
Duplicate NHS number[a] (Keep 1st card only)	1947	406 984

97 remaining patients had no NHS number, so duplicates may not have been excluded. However, this is only a small proportion of remaining samples (< 0.03% of samples)

There is some variability in whether IRT was reported in cases where IRT was initially raised above the analytical cut off but was insufficient for rechecks. Where IRT was reported in the CF result field, the result is included (15 samples), but where CF field was reported as insufficient (160 samples) the results are not included.

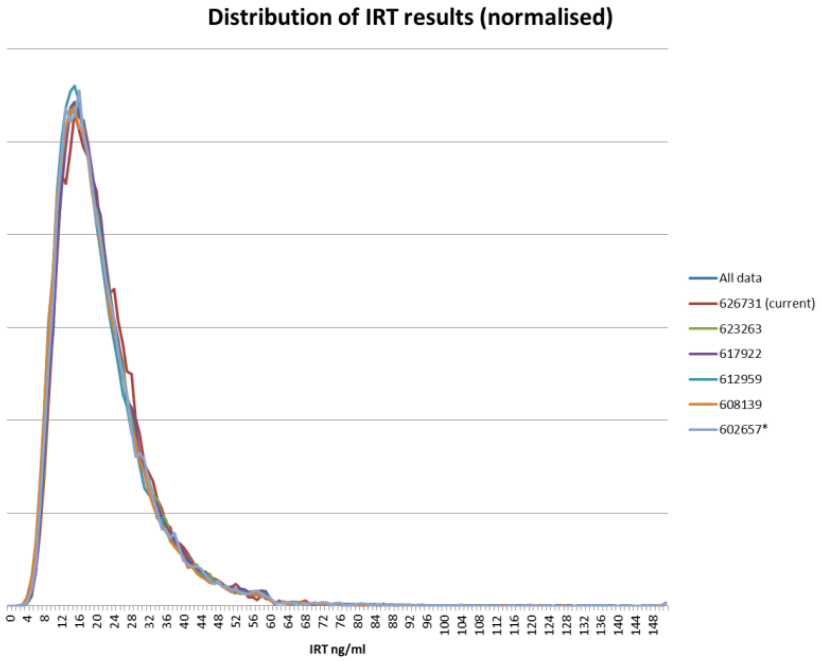
Kit lot number

Results were separated according to the kit lot used for analysis. Occasionally two different kit lots are in use at a single time; this occurs shortly after changing kit lot, where remaining old kits are used for repeats. These periods were excluded, so that results for each kit lot include only those results where the same kit lot was used for the main run and for any repeats.

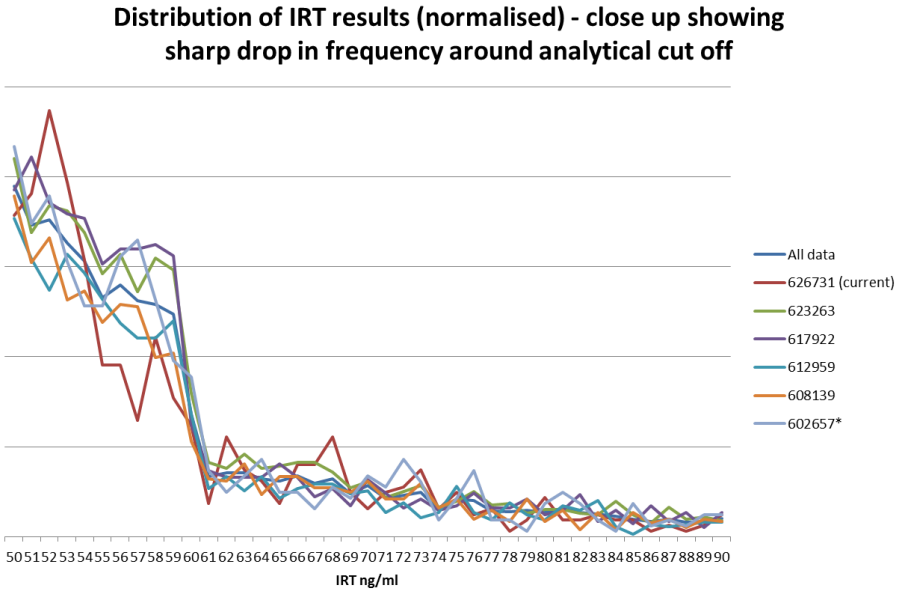
[a] Duplicate NHS numbers were removed by 1) sorting by NHS number then sample date 2) using formula IF(C2=C1,"DUPLICATE!","") to find duplicate cells (remove "DUPLICATE!" where NHS no blank, 3) Copy and paste values only 4) sort to remove duplicates.

Results

Distribution of IRT results



There is a sharp decrease in frequency around the analytical cut-off for IRT. This is probably due to systematic exclusion of samples above the analytical cut off as in many cases either (i) IRT was not reported as there was insufficient sample remaining for rechecks or (ii) the samples failed replicate checks. In addition, some regression to the mean is expected in repeated samples.



Centiles for IRT data

Calculated 99.5th centiles using > 30 000 data points for all lot numbers varied from 66.0 ng/ml to 72 ng/ml. The 99.5th centile using all data (> 400 000 data points) across 6 lot numbers was 69 ng/ml.

Calculated 99.9th centiles for individual kit lots varied from 96.5 ng/ml to 115.3 ng/ml.

Table – IRT centiles for all data, and for different lot numbers. Date ranges used for analysis are shown in brackets. 602657 was in use from July 2010 but only data from 1st Jan 2011 are included. 626731 is currently in use.

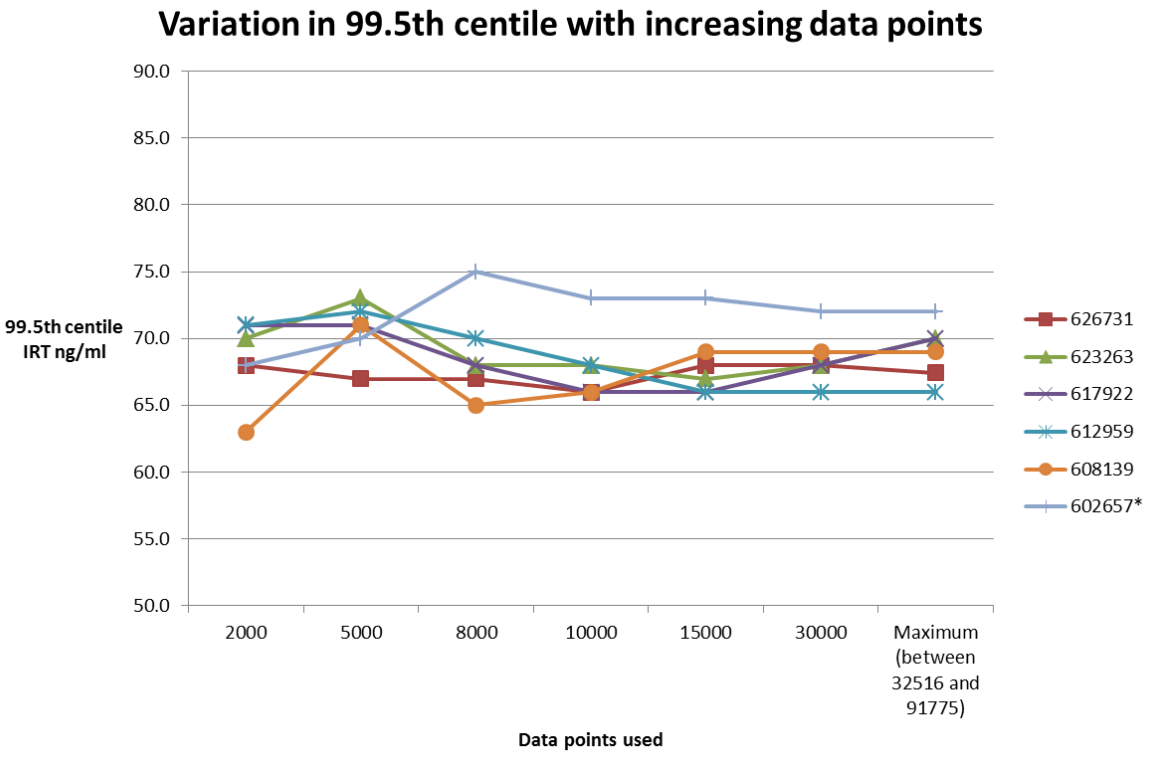
Centile	All data (Jan 2011 – May 2014)	626731 (Jan 2014 – May 2014)	623263 (April 2013 – Jan 2014)	617922 (Aug 2012 – April 2013)	612959 (Dec 2011 – July 2012)	608139 (Apr 2011 – Dec 2011)	602657 (Jan 2011 – April 2011)
10	11.0	11.0	11.0	11.0	10.0	10.0	11.0
20	13.0	13.0	13.0	13.0	12.0	12.0	13.0
30	15.0	15.0	15.0	15.0	14.0	14.0	15.0
40	17.0	17.0	17.0	17.0	16.0	16.0	17.0
50	**19.0**	**19.0**	**19.0**	**19.0**	**18.0**	**18.0**	**19.0**
60	21.0	22.0	21.0	21.0	20.0	21.0	21.0
70	24.0	25.0	24.0	24.0	23.0	23.0	24.0
80	28.0	28.0	28.0	28.0	27.0	27.0	28.0
90	34.0	35.0	35.0	35.0	34.0	33.0	34.0
99.5	**69.0**	**67.4**	**70.0**	**70.0**	**66.0**	**69.0**	**72.0**
99.9	**104.0**	**96.5**	**104.0**	**104.0**	**97.9**	**109.0**	**115.3**
Mean	21.1	21.7	21.6	21.5	20.5	20.7	21.1
Data points	406985	32516	91775	81946	75140	81369	32740

There is very close agreement between kit lots at normal IRT concentrations, with the median hardly affected by kit lot changes (either 18 ng/ml or 19 ng/ml). However, variation in the 99.5th centile was much larger. The mean also showed no correlation with the 99.5th centile (R^2 = 0.11). This suggests that the median and mean are of limited use in predicting the 99.5th centile form our data

Effect of number of data points on 99.5th centile

It has been suggested that at least 8000 data points are required to determine the 99.5th centile with a reasonable degree of statistical certainty. In some cases, the 99.5th centile derived from a small subset of data points showed good agreement with the 99.5th centile derived with the maximum number of data, for example lot number 626731 and 623263. However, in other cases (e.g. lot 612959), there were larger differences.

Data points	626731	623263	617922	612959	608139	602657*
2000	68.0	70.0	71.0	71.0	63.0	68.0
5000	67.0	73.0	71.0	72.0	71.0	70.0
8000	**67.0**	**68.0**	**68.0**	**70.0**	**65.0**	**75.0**
10000	66.0	68.0	66.0	68.0	66.0	73.0
15000	68.0	67.0	66.0	66.0	69.0	73.0
30000	68.0	68.0	68.0	66.0	69.0	72.0
Maximum	**67.4**	**70.0**	**70.0**	**66.0**	**69.0**	**72.0**
(maximum no of data points used)	(32516)	(91775)	(81946)	(75140)	(81369)	(32740)



The average error for each estimate of the 99.5th centile was compared to the 99.5th centile calculated using the maximum number of data points (assuming this to be the most accurate estimate). Using 10000 data points or fewer was worse than maintaining a constant 99.5th centile of 70 ng/ml (average error 1.6 ng/ml), at least for the kit lots analysed here.

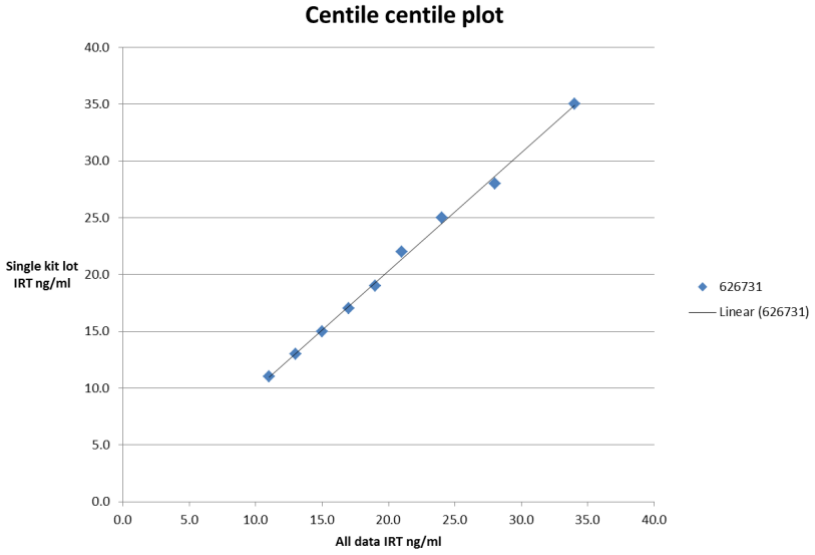
Table – Error for direct estimates of 99.5th centile compared to the 99.5th centile using maximum data points. Average error was calculated as the mean of absolute values.

Data points	626731	623263	617922	612959	608139	602657*	Average error
2000	0.6	0.0	1.0	5.0	-6.0	-4.0	2.8
5000	-0.4	3.0	1.0	6.0	2.0	-2.0	2.4
8000	-0.4	-2.0	-2.0	4.0	-4.0	3.0	2.6
10000	-1.4	-2.0	-4.0	2.0	-3.0	1.0	2.2
15000	0.6	-3.0	-4.0	0.0	0.0	1.0	1.4
30000	0.6	-2.0	-2.0	0.0	0.0	0.0	0.8

Using centile-centile plots to estimate the 99.5th centile

Plotting individual kit lot centiles against centiles derived with data from multiple kit lots has been suggested as a method for estimating assay bias. This could be used to estimate the 99.5th centile with a smaller number of data points.

For each kit lot, the 10th to 90th centiles were plotted and fitted with a straight line, which was used to estimate the 99.5th centile



The calculated 99.5th centiles with increasing data points for each kit lot were as follows:

Data points	626731	623263	617922	612959	608139	602657*
2000	71.3	72.3	69.4	73.5	68.9	68.0
5000	73.9	72.5	69.4	73.0	68.9	70.2
8000	74.1	70.8	69.4	71.5	68.9	69.1
30000	71.4	70.6	69.0	69.6	67.0	69.3
Maximum (maximum no of data points used)	71.4 (32516)	70.6 (91775)	70.6 (81946)	69.6 (75140)	68.2 (81369)	69.0 (32740)
Actual 99.5th centile	**67.4**	**70.0**	**70.0**	**66.0**	**69.0**	**72.0**

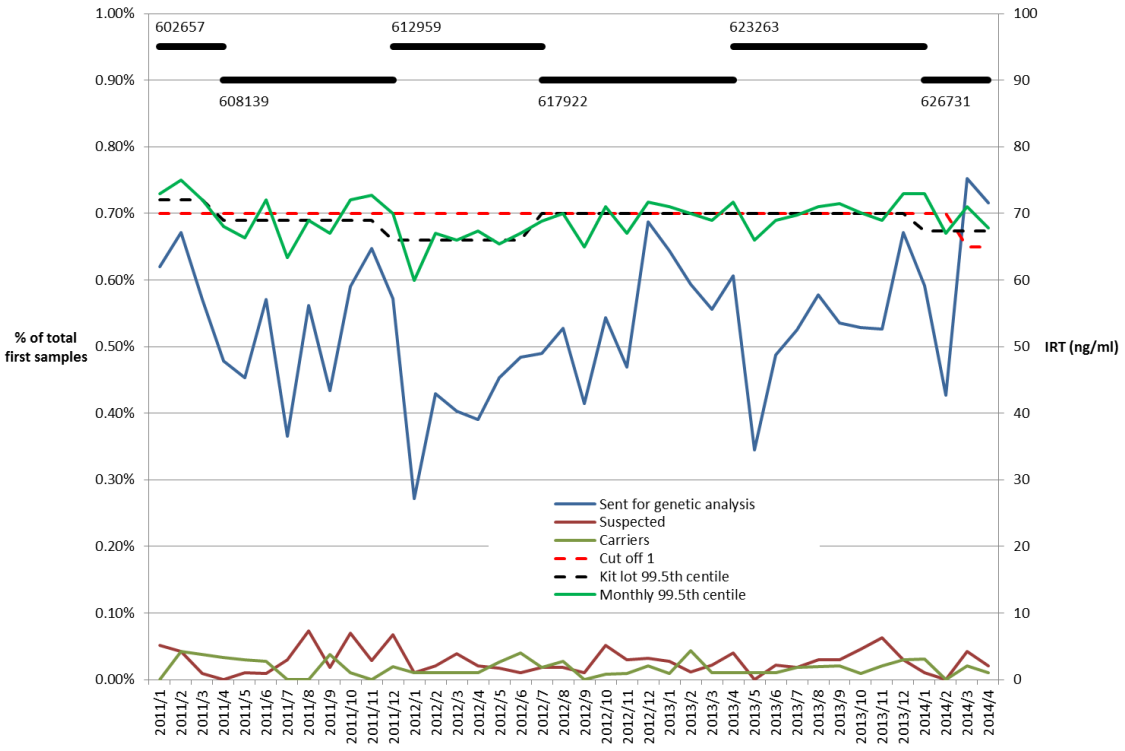
The difference between estimates of the 99.5th centile (using centile-centile plots) and the 99.5th centile calculated directly using all available data points is shown below. Average error was calculated as the mean of absolute values.

Data points	626731	623263	617922	612959	608139	602657*	Average error
2000	3.9	2.3	-0.6	7.5	-0.1	-4.0	3.1
5000	6.4	2.5	-0.6	7.0	-0.1	-1.8	3.1
8000	6.7	0.8	-0.6	5.5	-0.1	-2.9	2.8
30000	3.9	0.6	-1.0	3.6	-2.0	-2.7	2.3
Maximum	3.9	0.6	0.6	3.6	-0.8	-3.0	2.1

The average error from the 99.5th centile derived by centile-centile plots was larger than when the 99.5th centile was derived directly. This was true regardless of the number of data points used. This suggests that linear centile-centile plots are of limited use for determining the 99.5th centile.

Monthly monitoring of 99.5th centile and genetic analysis referral rates

Monthly 99.5th centiles and genetic analysis referral rates were monitored (Our population birth-rate is approximately 10000 samples per month). As expected, genetic referral rates (blue line) mirror changes in the observed monthly 99.5th centile (green line).



For kit lot 612959, the observed 99.5th centile was lower than assigned cut off 1. As a result, fewer samples were sent for genetic analysis (< 0.3% in January 2012).

For kit lot 626731 (our current lot), the observed 99.5th centile is slightly higher than the agreed buddy group B cut-off 1 set at 65 ng/ml. As a result, a larger number of samples have been sent for genetic analysis (> 0.7% in March and April 2014). Cut off 1 should ideally be increased to 67.4 or somewhere between 67 and 68 ng/ml.

The variability in monthly 99.5th centiles highlights that large changes in the 99.5th centile should not be made on < 10 000 data points. There is a risk of "overshooting" the 99.5th centile when insufficient data points are used for reliable estimation. For example, if the data from January 2012 had been used to derive the 99.5th centile, cut off 1 would have been set at a much lower concentration for kit lot 612959 (60 ng/ml vs. kit lot 99.5th centile of 66 ng/ml).

A practical solution for laboratories will small workloads would be to take the average of the old cut off 1 and the new derived value, and then evaluate this average cut off at a later date. For example, if cut off 1 was previously 70 ng/ml, but a month's data after changing kit lot suggest a 99.5th centile of 64 ng/ml, change cut off 1 to 67 ng/ml in the first instance, and evaluate again after another month to see the 99.5th centile still needs to be lowered again.

This cautious 'fine tuning' approach would avoid chasing large changes in the 99.5th centile due to the normal variation in the 99.5th centile seen with insufficient data points. Interesting, if this approach had been applied to the recent change in 99.5th centile at GOSH, cut off 1 would have been set at 67.5 ng/mL rather than 65 ng/ml, which show far better agreement with the 99.5th centile derived with all currently available data (67.4 ng/ml)

Discussion

Calculation of the 99.5th centile is complicated, with important decisions to make about which samples to exclude (for example, samples with a raised IRT that fail or are insufficient for rechecks). The previous analytical cut-off used can also affect future estimations of the 99.5th centile, as there is a marked decrease in the frequency of IRT concentrations slightly above the analytical cut-off. Careful thought needs to be given to the process to ensure samples are excluded and included appropriately.

Centile-centile plots have been suggested as a method to determine the 99.5th centile with fewer data points, however from our data this method appears to be less accurate than estimating the 99.5th centile directly, regardless of the number of data points used.

The median value showed very little variation between kit lots, and the average value showed no correlation with the 99.5th centile. This suggests that monitoring the median and mean are of little value in predicting changes in the 99.5th centile, beyond detecting gross changes in calibration.

The reliability of the 99.5th centile increases with the number of data points used.

The current 99.5th at GOSH is probably slightly too low, and the 99.9th centile too high. Values of 67 ng/ml and 104 ng/ml are probably more appropriate.

Conclusion

To monitor monthly 99.5th centiles, laboratories should include the first card for each patient, excluding contaminated cards, duplicate cards and cards that have failed replicate checks. Exclude cards if they are reported as insufficient for replicate checks.

It is advisable not to make large changes to cut off 1 based on less than 10 000 data points. If < 10 000 data points are to be used to derive a new 99.5th centile, use the average of the new value and the previous value to avoid overshooting the true 99.5th centile. Then re-evaluate after at least 10000 data points have been collected (more if possible)

We found that monthly monitoring of genetic analysis referral rates could provide a reasonable guide to the accuracy of cut-offs. Referral rates significantly below 0.5% support a decrease in cut off 1, while referral rates significantly above 0.5% support an increase in cut off 1.

We did not find good correlation between centile-centile plots and our 99.5th centile cut-off in all the 6 kit lots evaluated (over 3 years). The means or medians did not provide additional guides to possible changes in the 99.5th centile from our data.

Reference

1. R J Pollitt and A J Matthews NEWBORN SCREENING FOR CYSTIC FIBROSIS **Quality Management of the IRT Assay** Sheffield Children's Hospital 2006